Differential Pulse Polarographic and UV-Vis Spectrophotometric Study of Inclusion Complexes Formed by 1,4-Dihydropyridine Calcium Antagonists, Nifedipine and Nicardipine with β-Cyclodextrin

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Abstract
The formation of inclusion complexes of well-known 1,4-dihydropyridine calcium antagonists, such as nifedipine (NF) and nicardipine (NC), with β-cyclodextrin (βCD) was investigated by differential pulse polarography (DPP) and UV-vis spectrophotometry. The equimolar variation method indicated the formation of the NF-βCD (1:1, M:M) and a NC-βCD (1:1, M:M) inclusion complexes. Titrations using the DPP peak currents for NF and NC permitted one to determine formation constant values of (135 ± 20) M^{-1} and (357 ± 41) M^{-1} for NF-βCD and NC-βCD, respectively. For comparative purposes we have also applied phase solubility studies with spectrophotometric detection obtaining formation constant values of (129 ± 5) M^{-1} and (385 ± 19) M^{-1} for NF-βCD and NC-βCD, respectively. According to the DPP studies, we can postulate that the inclusion moiety were the nitroaromatic group, in the case of NF-βCD, and the phenyl group on 3-position of the 1,4-DHP, in the case of NC-βCD. The solubility of NF in water was increased about three times due to the formation of an inclusion complex with βCD. For NC the solubility was increased almost seven times.

Keywords: β-Cyclodextrin, Inclusion complexes, Differential pulse polarography, Nifedipine, Nicardipine

1. Introduction

The ability of cyclodextrins (CDs) and modified CDs to form inclusion complexes with a large variety of molecules are well known. This ability is based on the capability of CDs to provide a hydrophobic cavity in aqueous solution for both the hydrophobic guest molecule or moieties in the guest molecule [1]. This important feature makes it possible for CD to be used as biomimetic enzyme models [2, 3], drug delivery systems [4], electrode surface modifiers [5] and as a modifier of some properties in drugs, such as solubility and photoactivity [6, 7]. In the last years there are a lot of investigations related to the applications of CD with pharmaceuticals, food, cosmetics and personal care items reflecting the enhanced industrial importance of this compound [8].

1,4-Dihydropyridine calcium antagonists (1,4-DHP) exert their clinical effects by blocking the L class of voltage-gated calcium channels in a variety of tissues. These drugs are indicated in the basic treatment of essential arterial hypertension [9]. Nifedipine (NF) was the first of these type of drugs which have become available for clinical use as coronary vasodilator [10]. On the other hand, nicardipine (NC), is a 1,4-DHP structurally related to nifedipine with potent peripheral, cerebral and coronary arterial vasodilator that causes tenfold less myocardial depression in animals than NF and may provide important cardioprotective effects during ischemia [11]. Both of these compounds are slightly water soluble and light sensitive. NF is highly light-sensitive under both UV light and artificial daylight, generating the corresponding nitro and nitroso pyridine derivatives which are pharmacologically inactives [12]. NC is stable to artificial daylight but is sensitive to UV-light generating the pharmacologically inactive nitro pyridine derivative [13]. Consequently, both drugs show some undesirable properties from the point of view of their therapeutic applications, such as poor solubility in water and possible photochemical changes inducing loss in the therapeutic effect.

One approach in order to improve the solubility and the light resistance of the 1,4-DHP derivatives is the preparation of inclusion complexes of these drugs with CD. In fact, there are some studies showing that photochemical stability of 1,4-DHP was increased by the formation of inclusion complexes with βCD [7, 14–15]. In the case of isradipine, formation of inclusion complexes with methyl-βCD proved to increase twice the stability of the drug. Besides, photochemical stability of nimodipine in inclusion complexes with hydroxy-propyl-βCD (HP-βCD) and methyl-βCD was increased 30 and 70 times, respectively. Very recently [16], complex formation of NF with βCD and HP-βCD was studied, finding that solubility and dissolution rate of nifedipine were markedly enhanced by complexation. Furthermore, another recent study showed that inclusion

complexation between nicardipine hydrochloride and HP-βCD enhances the NC dissolution rate [17].

In the previous studies on the inclusion complexes of 1,4-DHP, the general method used were UV-vis spectrophotometry, HPLC (reverse phase) and HPTLC, differential scanning calorimetry, FTIR spectroscopy, X-ray diffractometry and scanning electron microscopy. In the present study, we used electrochemistry to investigate the inclusion complexation of NC and NF with βCD. We have taken advantage of the well-known electroactivity of these 1,4-DHP molecules to design a differential pulse polarographic procedure in order to obtain the formation constants and the host-to-guest ratio of the inclusion complexes, i.e., NC-βCD and NF-βCD, respectively. For comparative purposes, we have also performed an UV spectrophotometric assay.

2. Experimental

2.1. Reagents and Solutions

β-Cyclodextrin was obtained from Fluka and was used without prior purification. Nifedipine and nicardipine were supplied by Chile Laboratory (Santiago, Chile). All other reagents employed were of analytical grade.

A 1×10^{-2} M stock solutions of NF and NC were prepared in ethanol. Working solutions of both drugs were obtained by transferring a sample of adequate volume of stock solution into 10 mL volumetric flask containing an appropriate amount of cyclodextrin dissolved in Britton-Robinson buffer, pH 7.0. The mixed solution was diluted with Britton-Robinson buffer up to the final volume. Then, the solution was shaken thoroughly for 20 min. and allowed for equilibration at room temperature.

In order to avoid photodecomposition, all solutions containing NF and NC were thoroughly protected from light by working in amber vials or by wrapping the vials with an aluminum foil.

All the polarographic experiments were obtained after purging with N_2 for ten min. in the cell before each run. Temperature was kept constant at $(25 \pm 0.1)\,^{\circ}C$ in all experiments.

2.2. Apparatus

Polarographic curves were recorded on a totally automated BAS-100 voltammetric analyzer attached to a PC computer with proper BAS 100W version 2.3 software for total control of the experiments and data acquisition and treatment. A GCME polarographic stand with a dropping mercury electrode (DME) as the working electrode, a platinum wire as the counter electrode, and a Ag/AgCl electrode as the reference electrode was used.

Spectrophotometric measurements were carried out with an ATI Unicam Model UV3, UV-vis spectrophotometer using 1 cm quartz cell.

All pH measurements were carried out with a WTW microprocessor controlled standard-pH-ion meter pMX 3000/pH equipped with a glass pH-electrode SenTix 81. The standard solutions used for calibration were WTW 4.006, 6.865 and 9.180.

2.3. Procedures

Current titrations were carried out by keeping constant concentrations of NF or NC while varying concentration of βCD. The current titration equation is, as follows [18, 19]:

$$\frac{1}{[CD]} = K_f \frac{(1-A)}{1 - I/I_0} - K_f \tag{1}$$

where K_f is the apparent complex formation constant, I_0 and I are the peak currents in the absence and presence of βCD, respectively, [CD] is molar concentration of βCD and A is the proportional constant. The condition of using this equation is that a 1:1 association complex is formed and CD concentrations are much larger than the total concentration of the drug.

Solubility diagrams were obtained according to Higuchi and Connors [20]. Briefly, excess amounts of solid NF and NC (20 mg) were added to 10 mL of 0.1 M Britton-Robinson buffer of pH 7.0 containing various concentrations of βCD (0 to 15 mM). Samples were shaken for 24 h at 37 °C. Then, samples were stored for 4 days at 4 °C, and then filtered through a 0.45 μm membrane filter (Advantec MFS, Inc.). The NF and NC concentration in the filtrate was determined spectrophotometrically at 332 nm and 356 nm, respectively. The formation constant, K_f was calculated from the phase solubility diagrams according to the equation:

$$K_f = \frac{slope}{S_0(1 - slope)} \tag{2}$$

where S_0 is the solubility of NF or NC in the absence of βCD and slope means the corresponding slope of the phase solubility diagrams, i.e., the slope of the drug concentration versus CD concentration graph.

3. Results and Discussion

The interactions of a 1,4-DHP guest, such as NF or NC with the βCD host (Figure 1) were investigated by using DPP which is sufficiently sensitive to determine reliable data at low concentration of the electroactive guest. Typical DPP curves of 1.0×10^{-5} M NF and 0.5×10^{-5} M NC in 0.1 M Britton-Robinson buffer, pH 7.0, in the absence and presence of βCD are shown in Figure 2. The DPP curves for free NF or NC showed very well resolved cathodic peaks at $-$ 605 mV and $-$ 492 mV, respectively. These cathodic peaks are due to the four-electron, four-proton reduction of the

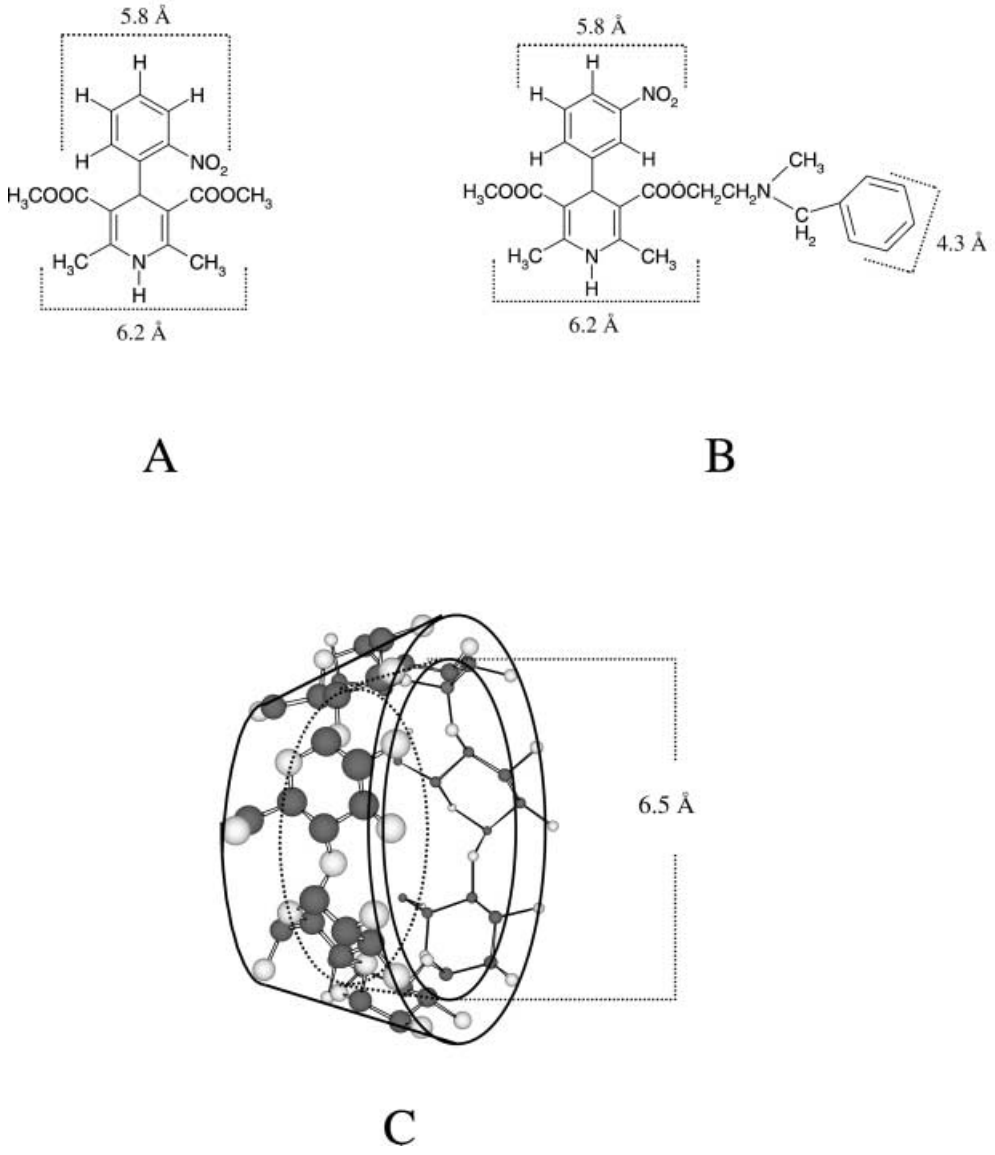

Fig. 1. Structural formulae of A) nifedipine, B) nicardipine and C) molecular structure of β-cyclodextrin, toroidal form (6.5 Å internal diameter).

nitroaromatic group to yield the hydroxylamine derivative. A more extensive study of the polarographic behavior of both drugs have been previously reported by us (12,13). Furthermore, as can be seen in Figure 2, the addition of βCD to the aqueous solution of both drugs causes changes in the polarograms. In the case of NC, with the increase of the amount of βCD the cathodic peak current (I_{PC}) decreased. On the other hand, in the case of NF the cathodic peak potential (E_{PC}) shifted negatively and $I_{P,C}$ decreased. These results are ascribed to the formation of the inclusion complexes according to the equilibrium:

$$\text{Guest} + \beta\text{CD} \leftrightarrow \text{Guest-CD} \quad (3)$$

wherein Guest-CD stands for the inclusion complex of NF or NC and βCD. The decrease of the peak current is due to a diminution of the apparent diffusion coefficient of the guest, forming inclusion complexes with βCD, compared to that of the free guest. The change in the E_{PC} reveals that electroreduction of NF molecules could be more difficult when they were included into the cavity of βCD. According to the above results, we can summarize that the effect of the guest concentration on the parameters I_{PC} and E_{PC} are different for NC and NF. That is, if the guest was NC then only I_{PC} varied but if the guest was NF then both I_{PC} and E_{PC} varied. Presumably, this difference is a consequence of a different insertion of the guest molecules. NF molecule have only one

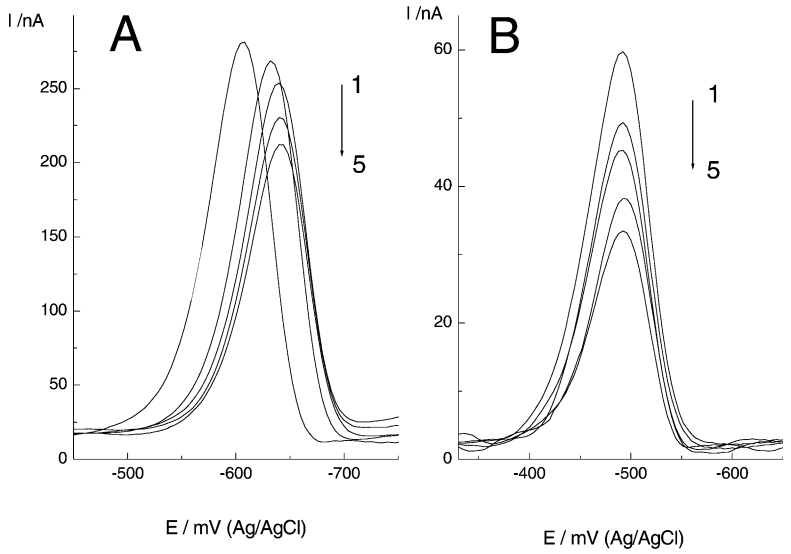

Fig. 2. DPP Curves for A) 1×10^{-5} M nifedipine in Britton-Robinson 0.1 M (pH 7.0) in absence (1) and presence of 2) 2.5, 3) 4.0, 4) 5.0, 5) 7.5 mM βCD. B) 5×10^{-6} M of nicardipine in Britton-Robinson 0.1 M (pH 7.0) in absence (1) and presence of 2) 2.0, 3) 3.5, 4) 5.0, 5) 9.0 mM βCD. Potential scan rate 4 mV s^{-1}, pulse amplitude 50 mV, pulse width 50 ms.

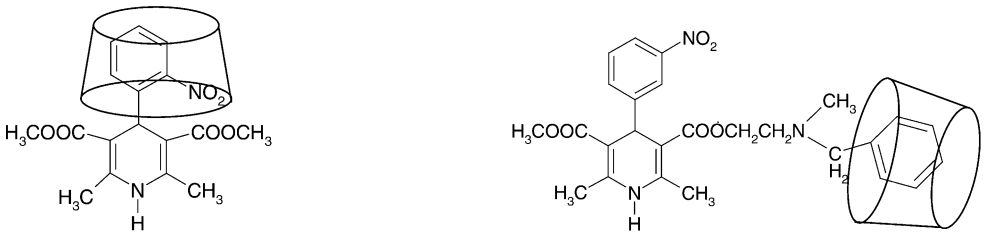

Fig. 3. Proposed structures of the inclusion complexes of nifedipine-β-cyclodextrin and nicardipine-β-cyclodextrin.

clear hydrophobic site, i.e., the nitroaromatic group, but the NC molecule has two hydrophobic sites, i.e., the nitroaromatic group and the phenyl group on 3-position of the 1,4-DHP. In Figure 3, we show graphically possible two different modes of insertion for NC and NF. As a consequence of insertion of NF, its electroactive group is located inside the βCD molecule. Hence, the CD creates a hydrophobic microenvironment that makes thermodynamically more difficult the reduction process of the nitroaromatic group. Clearly NF is bound on the reducible nitroaromatic moiety and this is reflected in the fact that the corresponding DPP peak potentials shift to more negative values as the βCD concentration increases. Importantly, another contribution to the complex formation is a steric effect. Thus, the size of both nitroaromatic and phenyl group (Fig. 1) is suitable for penetration the cavity of βCD (internal diameter of 6.5 Å).

The stoichiometry of the complex was determined by using the equimolar variation method (Job plot) [21], based on the difference in UV-vis absorbance, ΔA ($\Delta A = A_0 - A$), of NF or NC in the presence (A) and absence (A_0) of βCD. A series of solutions, in which the total concentration of host and guest species was maintained constant, and the mole fraction, χ, of the guest varied between 0 and 1 were prepared. Also, an equimolar aqueous solution of each βCD concentration was used as blank. A plot of $\Delta A[\mathrm{NF}]_t$ and $\Delta A[\mathrm{NC}]_t$ versus the mole fraction of NF and NC, respectively, is shown in Figure 4 (Job plot). Both plots show maximum at $\chi \approx 0.5$, indicating that both the NF-βCD and NC-βCD complex followed a 1:1 stoichiometry.

Considering that the 1:1 type inclusion complexes formed for NF and NC show decrease of peak currents with the increase of concentrations of βCD, we can apply the current titration procedure in order to calculate the formation constants. According to this procedure, we have obtained straight lines for the variables described in Equation 1 (Fig. 5). For NF and NC, these lines are described by Equations 4 and 5, respectively, obtained by the least squares linear regression fit:

$$1/[\beta\mathrm{CD}] = 92.71/(1 - I/I_0) - 1.35 \times 10^2 \quad (4)$$

$$1/[\beta\mathrm{CD}] = 167.8/(1 - I/I_0) - 3.57 \times 10^2 \quad (5)$$

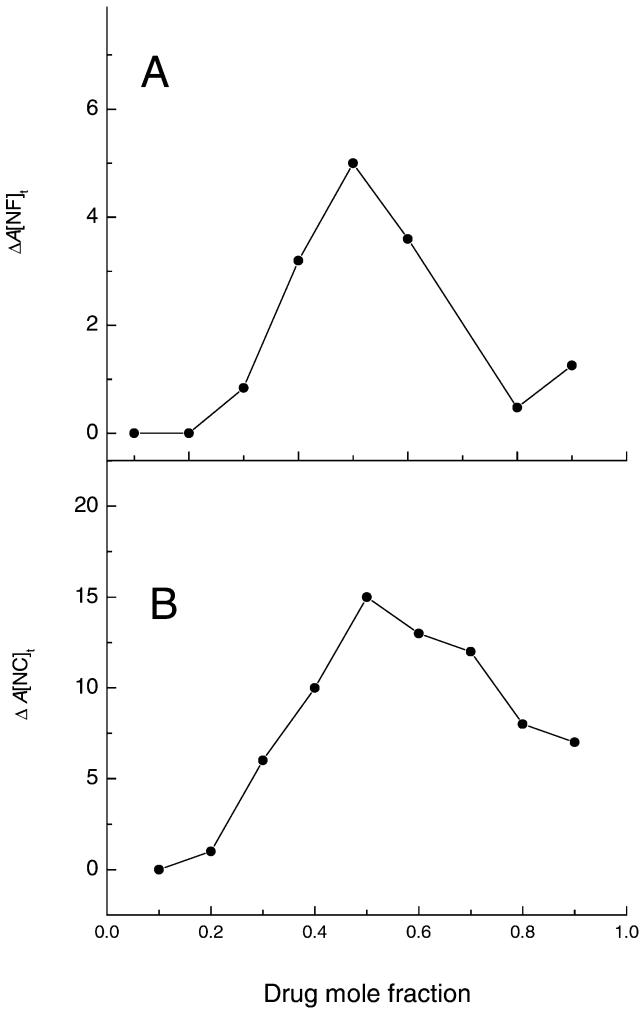

Fig. 4. Continuous variation plot (Job plot) of the absorption change in dependence of the A) nifedipine and B) nicardipine mole fraction.

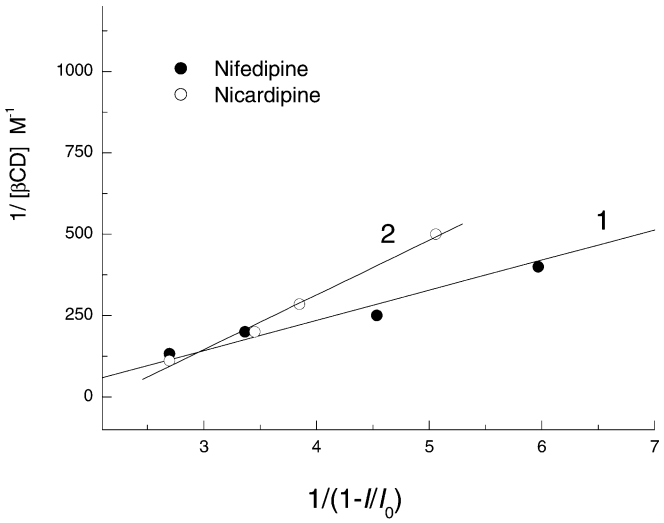

Fig. 5. Plot of 1/[CD] versus $1/(1 - I/I_0)$ for 1) nifedipine and 2) nicardipine.

with correlation coefficients of 0.9974 and 0.9951 for NF and NC, respectively.

Consequently, the results revealed that formation constants (K_f) for the inclusion complexes of NF or NC and βCD are equal to (135 ± 20) M^{-1} and (357 ± 41) M^{-1} for NF-βCD and NC-βCD, respectively, as calculated from the ordinate intercepts.

The formation of inclusion complex between NF or NC and βCD was further confirmed by spectrophotometric experiments. The UV spectra of NF and NC in the absence and presence of βCD are shown in Figure 6. Upon addition of βCD the absorbance of all bands of NF and NC increased. Moreover, the wavelengths of the absorption bands remain practically unaltered. When the concentration of βCD was increased to approximately 14 mM, the change of the absorbance became small at high concentrations of βCD and tended to saturation, suggesting that both NF and NC were included completely in βCD. Phase solubility analysis involves examination of the effect of a solubilizer, i.e., βCD on a solute, i.e., NF and NC. In our experiments, the UV-vis spectra show clearly that the solubility of the drug increased with the increase of the βCD concentration. The phase solubility profiles obtained for both NF-βCD and NC-βCD (Fig. 7) followed that for an A_L-type system [20]. This kind of curves are indicative of the formation of soluble inclusion complex and the corresponding formation constant (K_f) can be calculated by using the Equation 2. We have calculated the K_f values of (129 ± 5) M^{-1} and (385 ± 19) M^{-1} for NF-βCD and NC-βCD, respectively. Both results suggest that the inclusion complexes are formed and from the calculated values of K_f it follows that both inclusion complexes are relatively stable.

In Table 1 are summarized the polarographic and spectrophotometric K_f values determined in the present work and compared with some recently published related results.

Table 1. Formation constants (K_f) for nifedipine and nicardipine inclusion complexes with β-cyclodextrin determined in this work and its comparison with some related values in the literature.

Inclusion complex	K_f (M^{-1})		
	Polarography	Spectrophotometry	Spectrophotometry (other works)
NF-βCD	135 ± 20	129 ± 5	121.9 [16]
NC-βCD	357 ± 41	385 ± 19	49.26 ± 1.81 [17] [a]

[a] This value was determined for nicardipine hydrochloride.

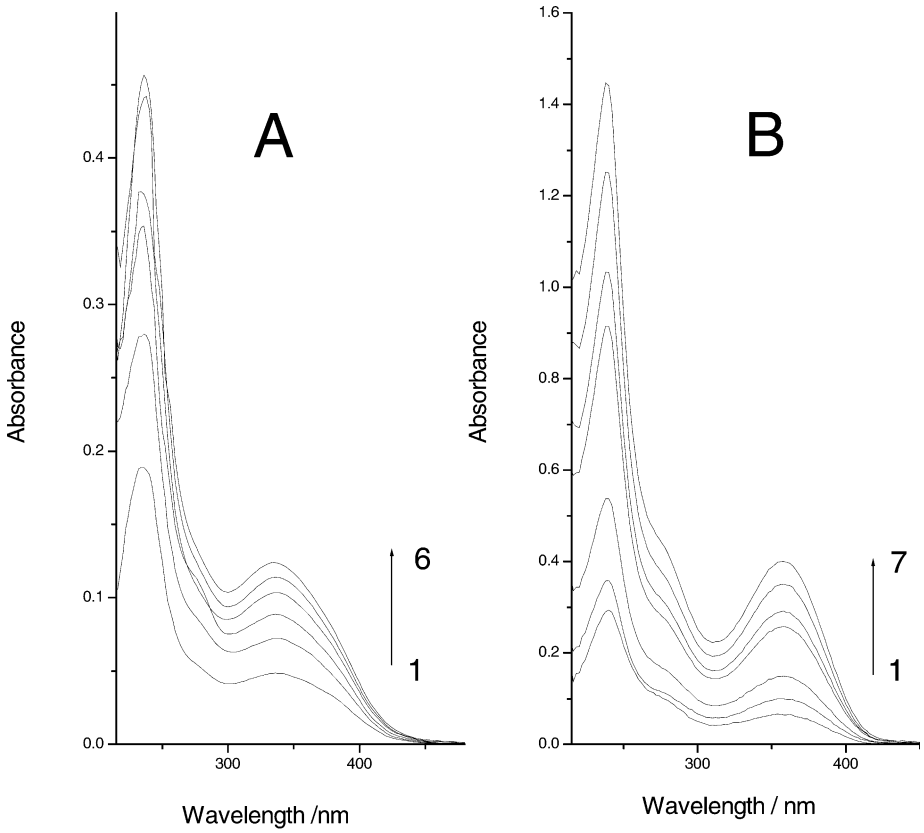

Fig. 6. A) Absorption spectra of nifedipine and 1) 0, 2) 4, 3) 6, 4) 8 5) 10, 6) 12 mM βCD. B) Absorption spectra of nicardipine and 1) 0, 2) 3, 3) 5, 4) 7, 5) 8, 6) 10, 7) 13 mM βCD.

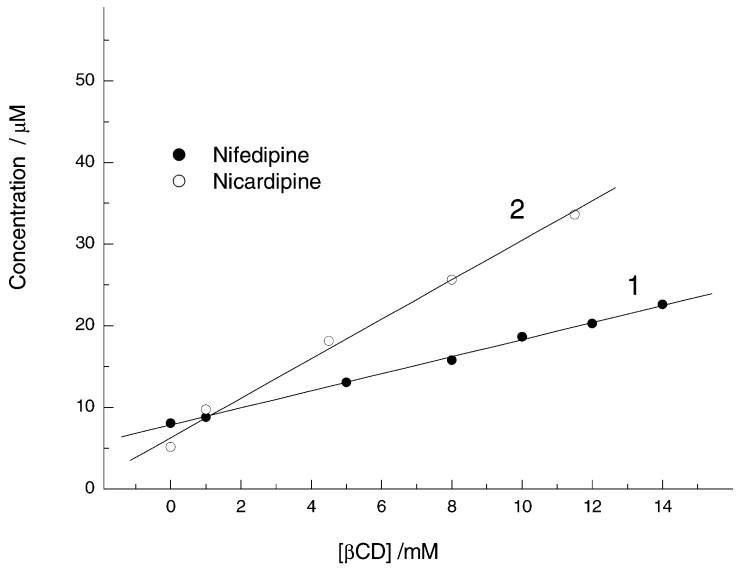

Fig. 7. Phase solubility diagrams for 1) nifedipine and 2) nicardipine against increasing concentrations of βCD.

The electrochemically and spectrophotometrically determined formation constants are very close to each other validating our results. Consequently, the polarographic approach can be recommended as a very good alternative to determining the formation constant of inclusion complexes of βCD. Furthermore, there is very good agreement between our results and the recently published K_f value for the NF complex of βCD [16]. In the case of the results for

NC, the agreement is not very good because in reference 17 the guest drug was the hydrochloride derivative which, clearly, is more soluble than the parent compound.

From the above results we can conclude that the solubility of NF was increased about three times due to the formation of an inclusion complex with βCD. For NC the solubility was increased almost seven times.

4. Conclusions

We have demonstrated by two independent methods that nifedipine and nicardipine forms a 1:1 inclusion complex with β-cyclodextrin. Furthermore, we have calculated formation constants values of $(135 \pm 20)\ M^{-1}$ and $(129 \pm 5)\ M^{-1}$ for nifedipine and $(357 \pm 41)\ M^{-1}$ and $(385 \pm 19)\ M^{-1}$ for nicardipine from electrochemical and spectrophotometric measurements, respectively. The solubility of nifedipine was increased about three times and nicardipine almost seven times due to the formation of inclusion complexes with βCD.

5. Acknowledgement

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6. References

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